ABSTRACT

The thesis entitled "**Total synthesis of Pladienolide B and its analogues**" has been alienated into FIVE chapters**. Chapter I** describes a brief introduction to (a) Natural product hybrids (b) Diverted Total Synthesis (DTS). **Chapter II** describes the total synthesis of pladienolide B. **Chapter III** describes the synthesis of truncated analogues of pladienolide B. **Chapter IV** describes the stereoselective syntheses of spiroacetals attenols A and B. **Chapter V** describes the synthesis of neolignan honokiol analogues.

CHAPTER I:

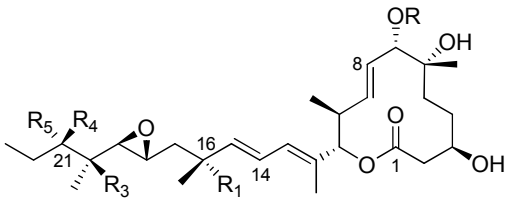
This Chapter describes a brief introduction to (a) Natural Prodcuct Hybrids; (b) Diverted Total Synthesis (DTS).

CHAPTER II:

This Chapter describes the "Total synthesis of pladienolide B".

Pladienolides are 12-membered macrocyclic polyketides isolated from *Streptomyces platensis* Mer-11107 by way of an assay targeting that inhibit cell signalling pathways in a tumor-specific microenvironment. These novel polyketides contain a 12-membered macrocyclic core, a 12-carbon diene side-chain, up to 11 stereocenters and an *E*-olefin embedded in the macrocyclic ring (Figure 1.1). Six of the seven pladienolides were reported to inhibit hypoxia-induced gene expression of vascular endothelial growth factor (VEGF) in U251 human glioma cells. The most active pladienolides B (**2**),C (**3**) and D (**4**) had IC_{50}-values in the

low nanomolar range. Pladienolides have a unique mode of mechanistic action involving binding to the splicing factor SF3b an essential component of the spliceosome. Notably pladienolides B and D also caused *in vivo* tumor regression in several human cancer xenograft models. It has recently been reported that a derivative of pladienolide B has entered human clinical trials for cancer. The absolute configuration of **2** and **3** were recently determined by NMR and synthetic studies. The stereochemistry of the other congeners is unknown.



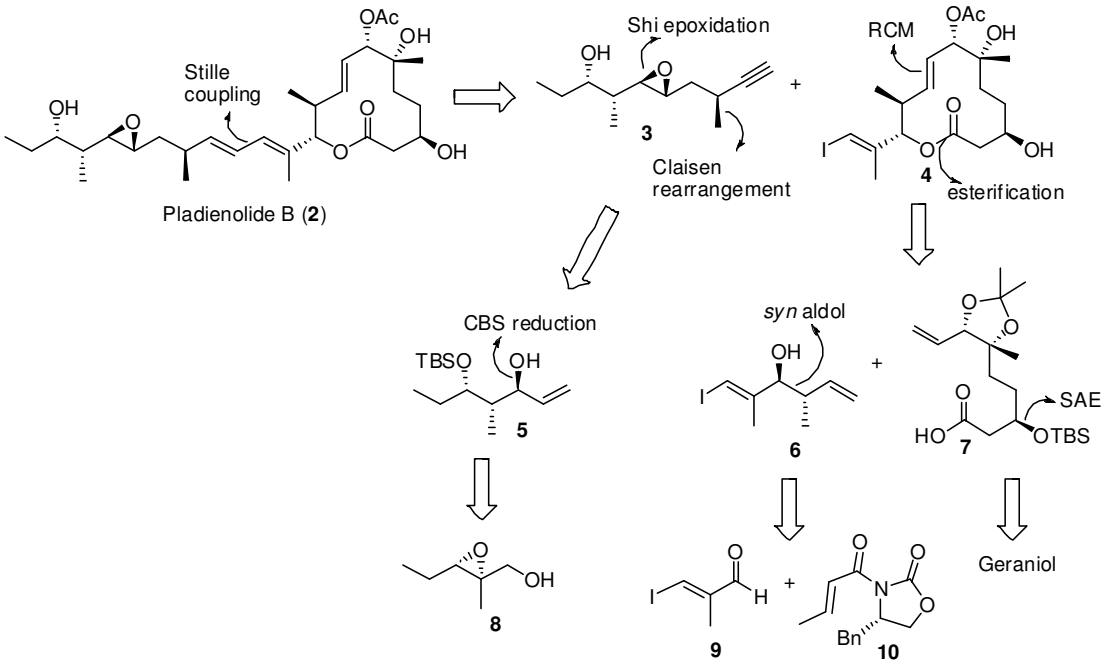
Pladienolide A (**1**): R = H, R_1 = H, R_2 = H, R_3 = H, R_4 = OH
B (**2**): R = Ac, R_1 = H, R_2 = H, R_3 = H, R_4 = OH
C (**3**): R = Ac, R_1 = H, R_2 = H, R_3 = R_4 = =O
D (**4**): R = Ac, R_1 = OH, R_2 = H, R_3 = H, R_4 = OH
E (**5**): R = Ac, R_1 = H, R_2 = OH, R_3 = H, R_4 = OH
F (**6**): R = H, R_1 = OH, R_2 = H, R_3 = H, R_4 = OH
G (**7**): R = H, R_1 = H, R_2 = OH, R_3 = H, R_4 = OH

Figure 1.1: Structures of pladienolides A-G

Present Work:

The challenging architecture, in conjunction with the novel biological profile of pladienolide B prompted the total synthesis. From the retrosynthetic perspective (Scheme 1.1) Stille coupling was employed to append the side-chain **3** to the macrocyclic core **4**, offering the *E,E*-diene system. The macrocyclic core **4** was synthesized by sequential esterification followed by ring closing metathesis (RCM) of hydroxy vinyl iodide (HVI) **6** and acid **7.** The HVI **6** could be constructed by coupling vinyl iodo aldehyde **9** and Evan's amide **10,** whereas the acid **8** realized

from commercially available geraniol. The side-chain moiety **3** was planned from the known epoxy alcohol **8** through the intermediate **5**.

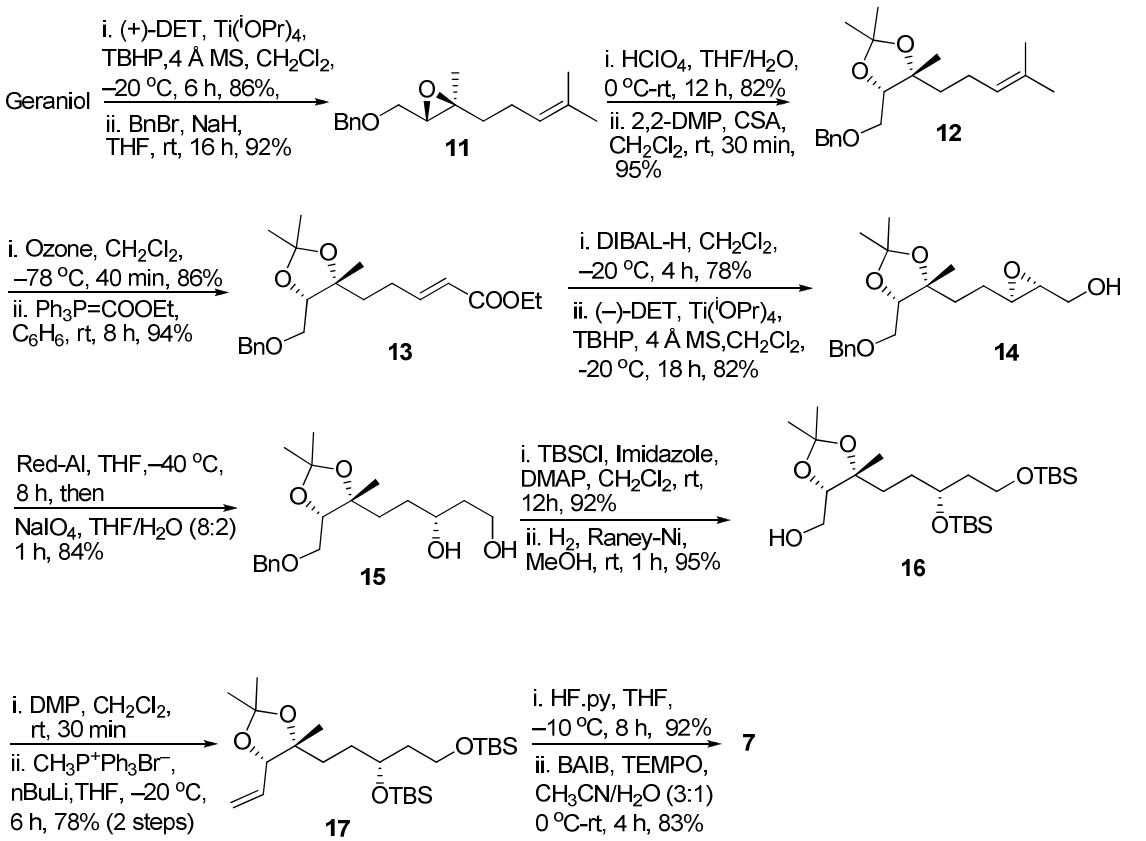


Scheme 1.1: Retrosynthetic analysis of pladienolide B, **2**.

Synthesis of acid (7):

Geraniol was successively subjected to Sharpless asymmetric epoxidation followed by benzylation of alcohol with BnBr, NaH in THF afforded **11** in 79% yield. Opening of epoxide functionality using perchloric acid to furnish diol and the resultant diol was protected as an acetonide (2,2-DMP, cat. CSA in CH_2Cl_2) to provide **12** in 78% yield. Ozonolysis of trisubstituted olefin in **12** produced aldehyde, which on two carbon homologation using Ph_3P=CHCOOEt offered *a, β*-unsaturated ester **13**. Reduction of ester functionality in **13** with DIBAL-H followed by asymmetric epoxidation using Sharpless conditions afforded epoxy

alcohol **14** in 64% yield. Reductive opening of epoxy alcohol **14** to 1,3-diol **15** was achieved with Red-Al in THF at −40 °C in 84% yield. Disilylation of 1,3-diol **15**, followed by debenzylation with Raney-Ni furnished primary alcohol **16** in 84% yield. One carbon homologation of **16** to **17** was obtained in two steps (oxidation followed by Wittig olefination). The selective cleavage of primary silyl ether in **17** followed by oxidation with BAIB/TEMPO provided acid **7** in 77% yield (Scheme 1.2).

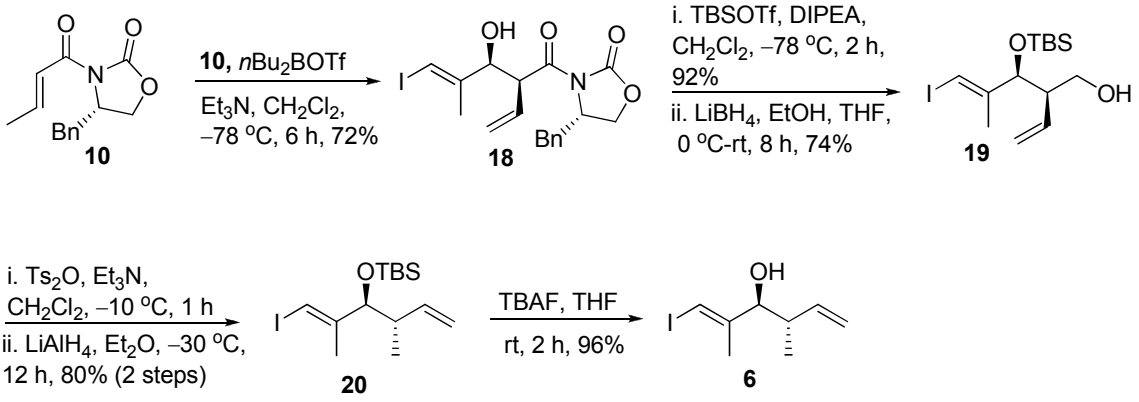


Scheme 1.2:

Synthesis of hydroxy vinyl iodide (HVI, 6):

Synthesis of **6** was started with *n*Bu$_2$BOTf mediated *syn*-aldol condensation of aldehyde **9** with oxazolidinone amide **10** to afford the

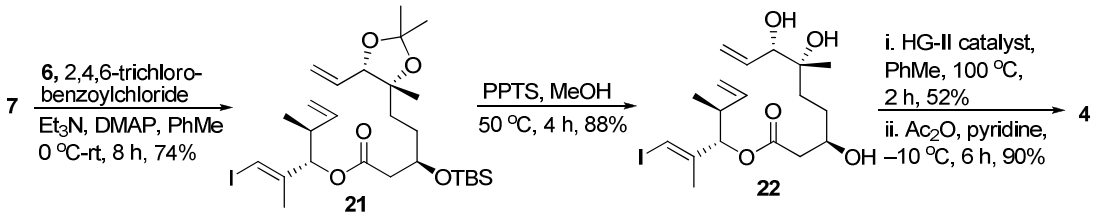
adduct **18** in 72% yield with good diastereoselectivity (>25:1). Silylation of **18** followed by reduction of chiral auxiliary with $LiBH_4$ to yield primary alcohol **19** in 68% yield. Deoxygenation of alcohol in **19** was accomplished in two steps *via* tosylation and $LiAlH_4$ reduction to attain **20** in 80% yield. TBAF mediated desilylation of **20** gave HVI **6** in 96% yield (Scheme 1.3).



Scheme 1.3

Synthesis of macrocyclic core (4):

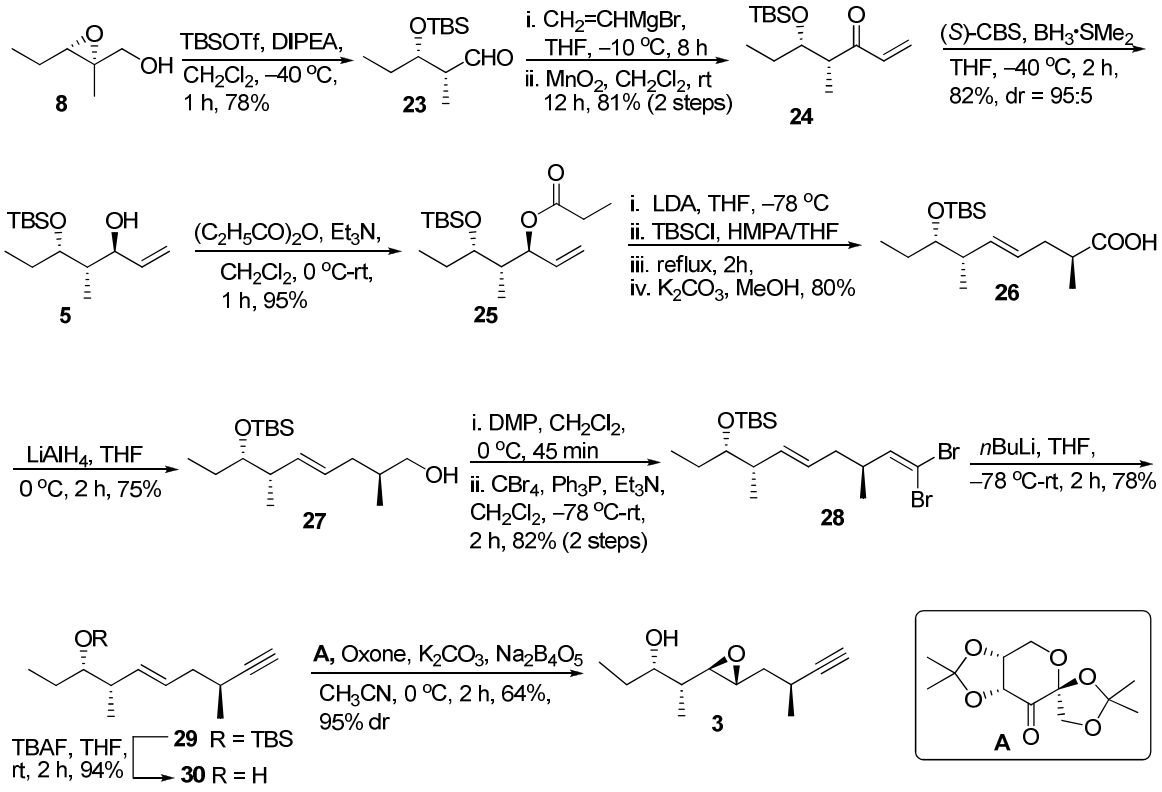
The construction of the macrolactone core **4** achieved as shown in Scheme 1.4. Esterification of acid **7** with HVI **6** was realized under Yamaguchi conditions to furnish fully functionalized ester **21** in 74% yield. Hydrolysis of isopropylidine group and TBS ether using PPTS/MeOH offered corresponding triol **22** in 88% yield. Triol **22** underwent RCM with HG-II catalyst in the presence of 1,4-benzoquinone provided macrolactone which was subjected to regioselective acetylation with Ac_2O in py at –10 °C was very efficient to furnish acetate **4** in 47% yield.



Scheme 1.4:

Synthesis of side-chain moiety (3):

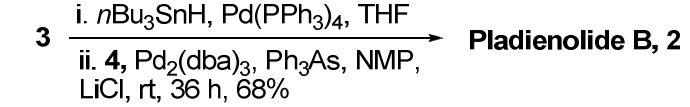
The side-chain **3** was synthesized starting from known epoxy alcohol **8** as shown in Scheme 1.5. TBSOTf mediated intramolecular hydride transfer in epoxy alcohol **8** gave silyloxy aldehyde **23** (78%). The vinylmagnesium bromide addition onto aldehyde **23** followed by MnO_2 oxidation afforded enone **24** in 81% yield. Enantioselective reduction of enone **24** with (*S*)-CBS reagent and $BH_3.SMe_2$ gave allylic alcohol **25** (82%, *dr* = 95:5). Propionylation ($(C_2H_5CO)_2O$, Et_3N in CH_2Cl_2) of alcohol **25** furnished ester **5** (95%) and subsequent Ireland–Claisen rearrangement with LDA and TBSCl in the presence of HMPA gave acid **26** with additional asymmetric carbon at C16 position. Reduction of acid functionality in **26** with $LiAlH_4$ provided primary alcohol **27** (75%). The Corey protocol was employed to synthesize the alkyne **29** via dibromide **28** in 82% yield. Desilylation of **29** using TBAF afforded the homoallylic alcohol **30** which was exposed to Shi epoxidation conditions produced epoxy alkyne **3**.



Scheme 1.5:

Synthesis of Pladienolide B (2):

With access to the side-chain **3** and macrolide **4**, we sought to couple these employing the Stille coupling. In the event, exposing the side chain fragment **3** to nBu$_3$SnH in the presence of Pd(PPh$_3$)$_4$ to afford unstable vinylstannane, which was immediately coupled with macrolactone **4** in the presence of Pd-catalyst, Ph$_3$As, LiCl in NMP afforded the desired pladienolide B (**2**) in 68% yield (Scheme 1.6).



Scheme 1.6:

In conclusion, a convergent synthetic route has been achieved efficiently. The synthetic highlights include Sharpless asymmetric epoxidation, Ireland-Claisen rearrangement, ring closing metathesis, Shi epoxidation and Stille coupling.

CHAPTER III:

This Chapter describes the "Synthesis of truncated analogues of pladienolide B".

Pladienolides A-G were isolated from *streptomyces platensis* Mer-11107 by way cell based assay that inhibit cell signaling pathways. Six of the seven pladienolides were reported to inhibit hypoxia-induced gene expression of VEGF in human glioma cells. Interestingly, pladienolides B, C and D all have the C7 acetyl group and showed good biological activity. Lack of this acetate decreased activity by more than two orders of magnitude as witnessed by pladienolides A, F and G (Table 1.1). Pladienolides B and D also caused *in vivo* tumor regression in several human xenograft models.

Table 1.1:

Compound	Anti-VEGF-PLAP activity IC_{50} (nM)	Anti-proliferative activity IC_{50} (nM)
Pladienolide B (**2**)	1.8	3.5
Pladienolide D (**4**)	5.1	6.0
Pladienolide C (**3**)	7.4	14.7
Pladienolide E (**5**)	65.2	146.8
Pladienolide A (**1**)	451.5	967.5
Pladienolide F (**6**)	2894.2	2595.2
Pladienolide G (**7**)	> 10000.0	> 10000.0

Our interest in pladienolide B and closely related congeners was prompted by the highly interesting biological profile displayed by this family of natural products. The highly potent *in vitro* and in vivo antitumor activity as well as the unique mechanism of action presented by this class of compounds makes pladienolide B a promising lead structure in the identification of novel anti-tumor agents. As part of our programme to design and synthesis of novel anti-cancer agents based on natural products, we selected new pladienolide B analogues as our target molecules. The truncated analogues of pladienolide B were designed with aromatic substituents in place of side-chain to know the side-chain importance in biological activity (Figure 1.2).

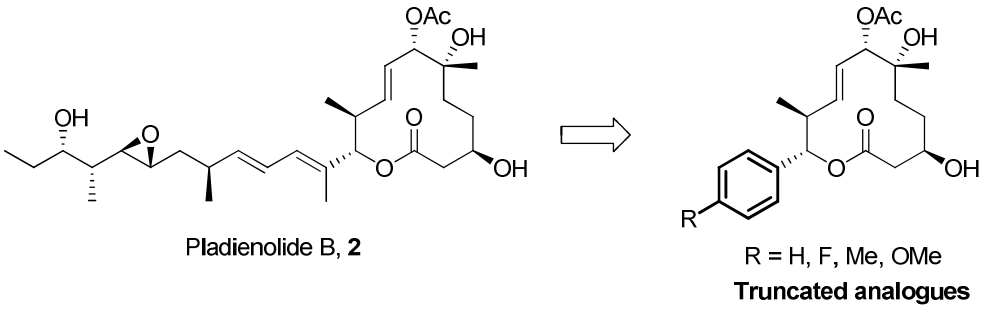
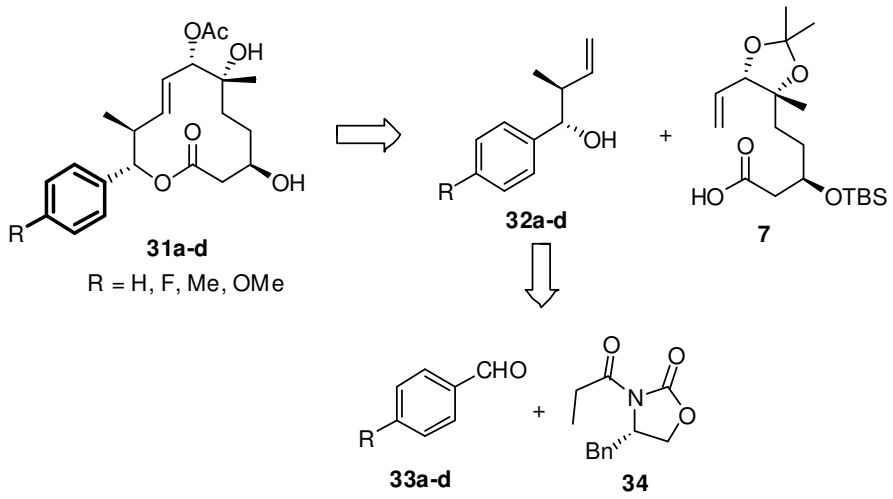


Figure 1.2:

Present Work:

From retrosynthetic analysis (Scheme 1.7) truncated analogues (**31a-d**) could be synthesized from alcohols **32a-d** and acid **7** by employing sequential Yamaguchi esterification and RCM reactions. Alcohols **32a-d** could be obtained from $MgCl_2$ catalyzed *anti*-aldol reaction between aromatic aldehydes **33a-d** and oxazolidinone **34** with ease.

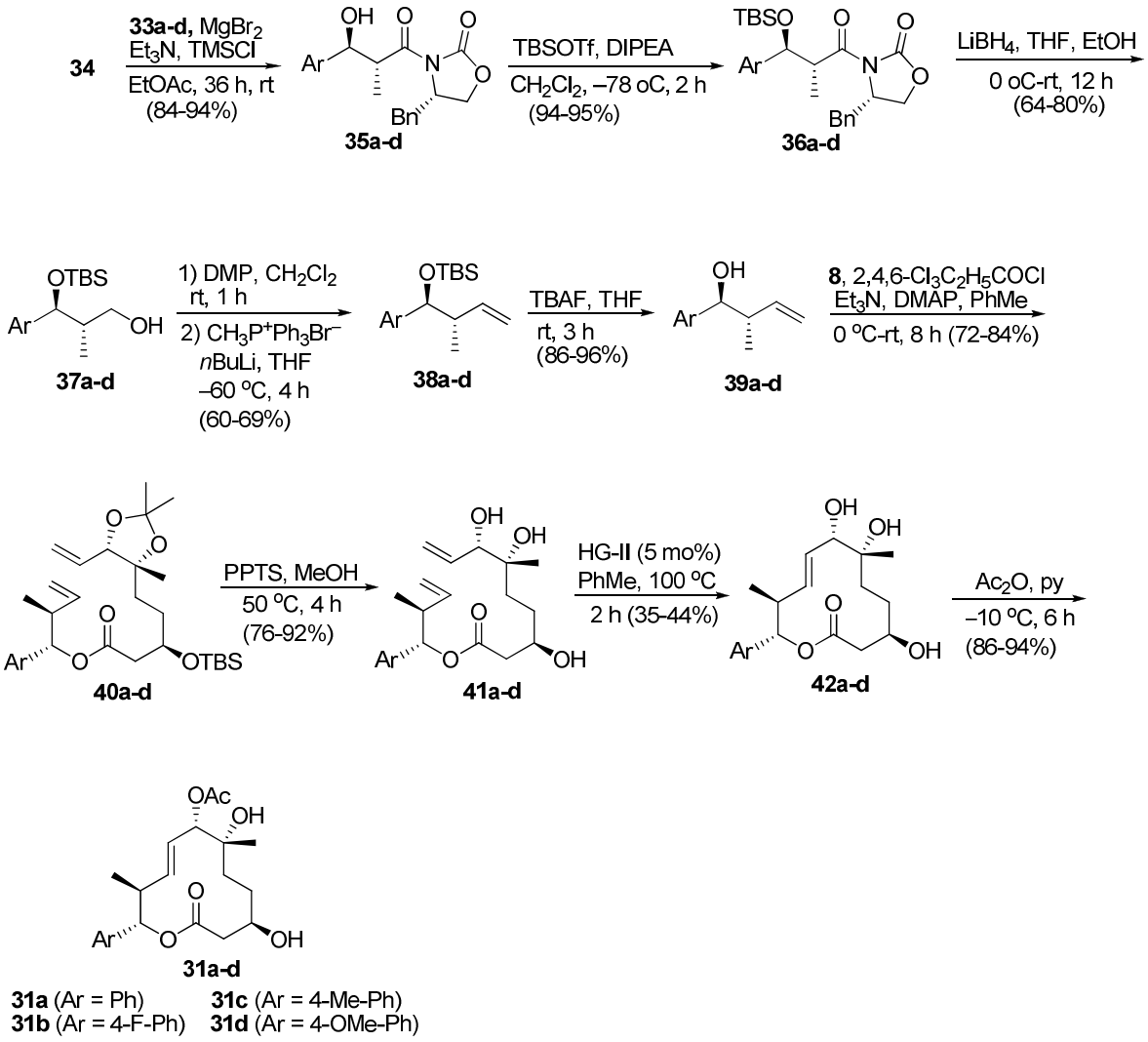


Scheme 1.7: Retrosynthetic analysis of pladienolide B analogues

Synthesis of truncated analogues of pladienolide B (31a-d):

The syntheses commenced with $MgCl_2$ *anti*-aldol reaction between oxazolidinone **34** and aromatic aldehydes **33a-d** to afford adducts **35a-d** in 84-94% yield. These adducts were silylated as TBS-ethers **36a-d** and removal of chiral auxiliary with $LiBH_4$ provided primary alcohols **37a-d**. One carbon homologation of 37a-d accomplished in two steps (oxidation and methylenation) gave olefins **38a-d** in 60-69% yield. TBAF mediated desilylation of **38a-d** provided homoallylic alcohols **39a-d** in 86-96% yield. Esterification of alcohols **39a-d** with previously synthesized acid **7** under Yamaguchi conditions afforded esters **40a-d** and under acid hydrolysis conditions provided triols **41a-d**. The central 12-membered lactones **42a-d** were formed in moderate yields through RCM using Hoveyda-Grubbs 2nd generation catalyst in the presence of 1,4-benzoquinone at reflux temperature from triols **41a-d**. Finally,

regioselective acetylation of more nucleophilic hydroxy group (C7) gave the desired truncated analogues of pladienolide B **43a-d** in 86-94% yield (Scheme 1.8).



Scheme 1.8:

In summary, syntheses of four truncated analogues of pladienolide B were achieved using $MgCl_2$ *anti*-aldol reaction, Yamaguchi esterification and RCM as key reactions. The biological evaluation of synthetic pladienolide B and designed truncated analogues were performed against

three cancer cell lines and showed that, the designed analogues inhibits tumor moderately.

CHAPTER IV:

This Chapter describes the "Stereoselective syntheses of spiroacetals attenols A and B".

Two novel isomeric etheral compounds by Uemura et al. from the dichloromethane-soluble fraction of the ethanol extract of the Chinese biovalve *Pinna attenuata* and named as attenol A (**43**) and attenol B (**44,** Figure 1.3). These bicyclic triols exhibit moderate cytotoxicity against P388 cells at IC_{50} 24 and 12 μg/mL, respectively. Attenols possess same molecular formulae but differ in the two hydroxy groups which participate in the spiroketal linkage. Attenol A comprises a [5,4] spiroketal unit and attenol B featuring a dioxa-bicyclo [3.2.1]octane unit. Attenol A features a spiroketal ring embedded with six stereocentres, a disubstituted (*Z*)-olefin and terminal olefin.

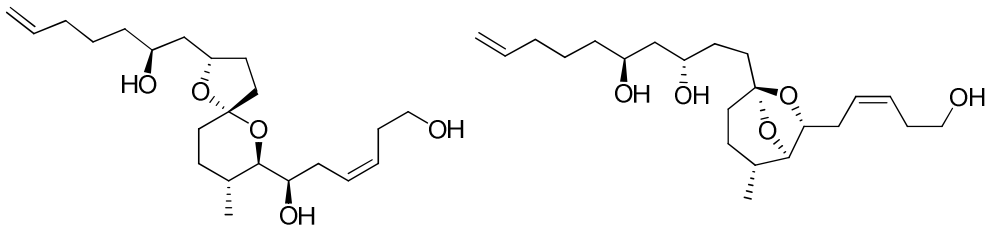
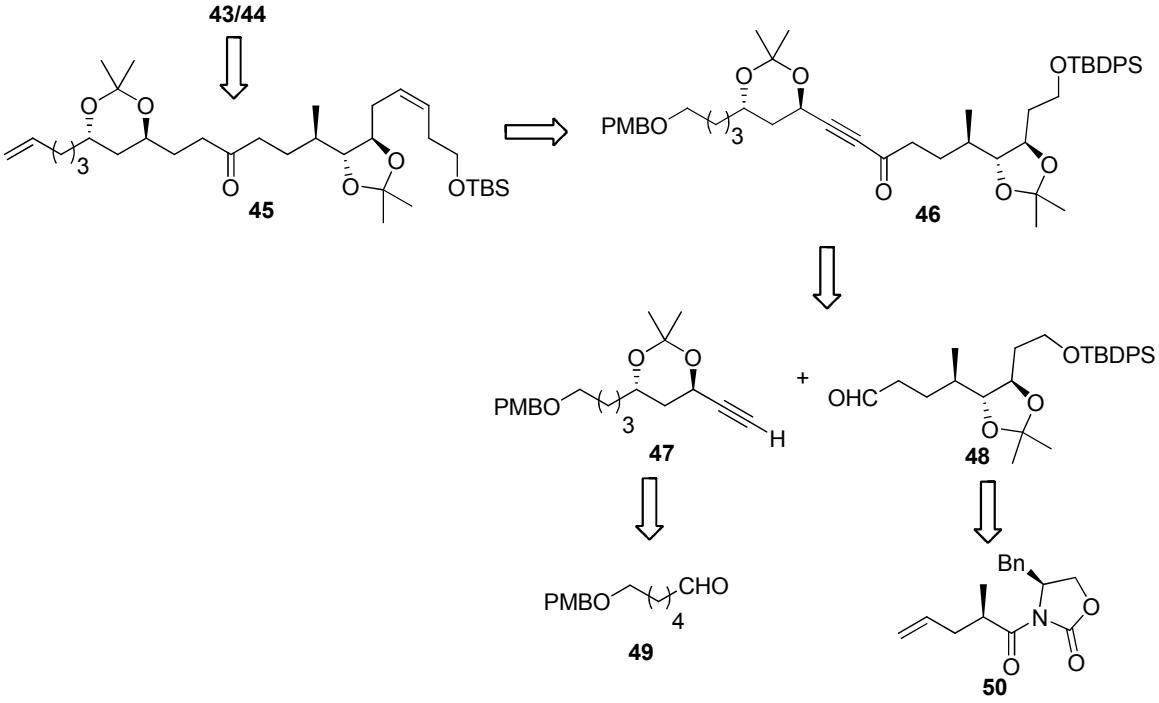


Figure 1.3:

Present Work:

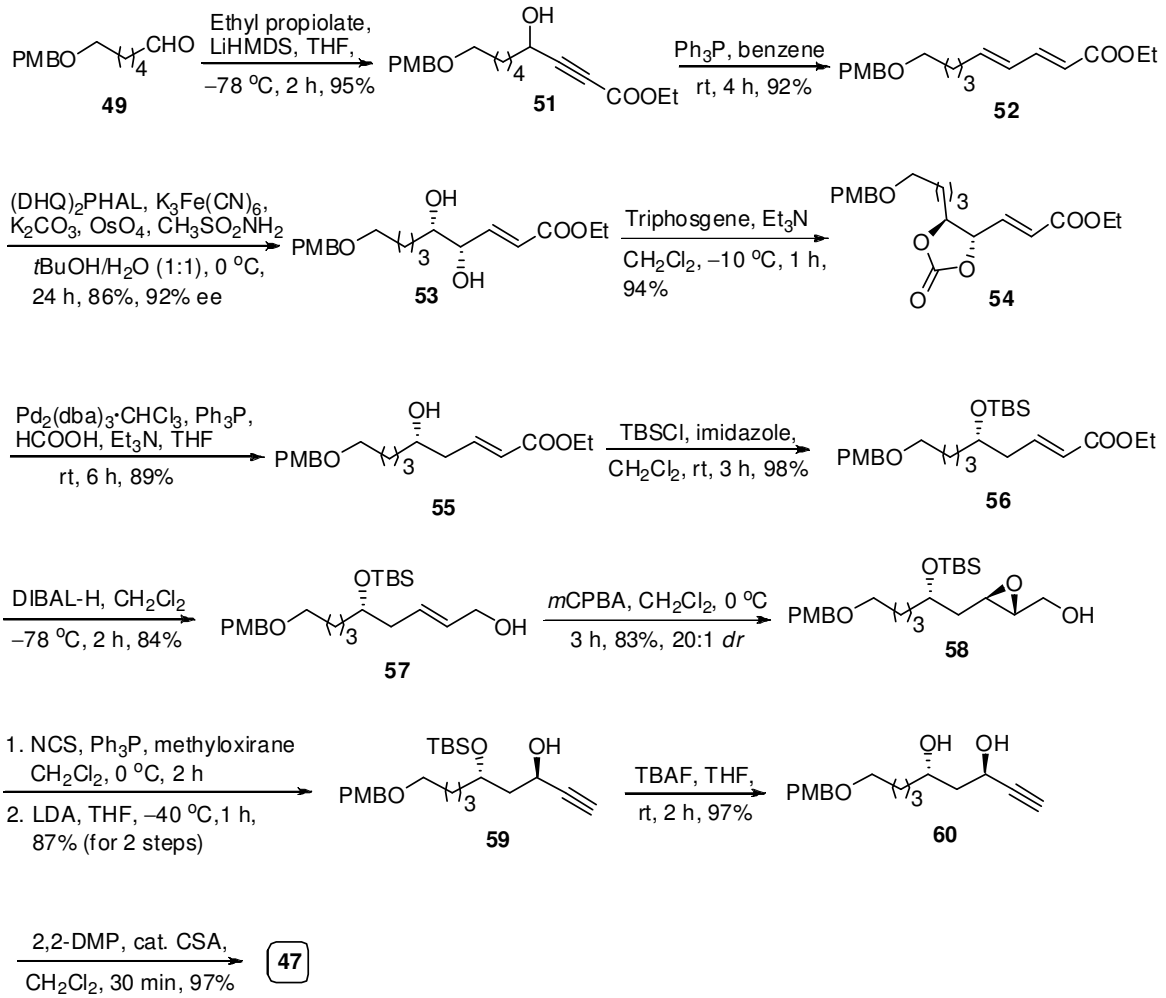
As shown in Scheme 1.9 attenols A and B were constructed from ketone **45** in one step by acid catalyzed spiroketalization. The advanced ketone **45** could be constructed from alkynone **46**, which in turn may be built from the alkyne **47** and the aldehyde **48**. The fragments **47** and **48** could be obtained from known aldehyde **49** and amide **50**, respectively.



Scheme 1.9: Retrosynthetic analysis of attenols A and B

Synthesis of alkyne 47:

Synthesis of terminal alkyne **47** commenced from the known aldehyde **49** in 11 steps as illustrated in Scheme 1.10. Treatment of aldehyde **49** with lithiated ethyl propiolate afforded the propargylic alcohol **51**. Exposure of γ-hydroxy ynoate **51** to the Lu protocol (PPh_3/benzene) provided dienoate **52** with 97% diastereocontrol of the

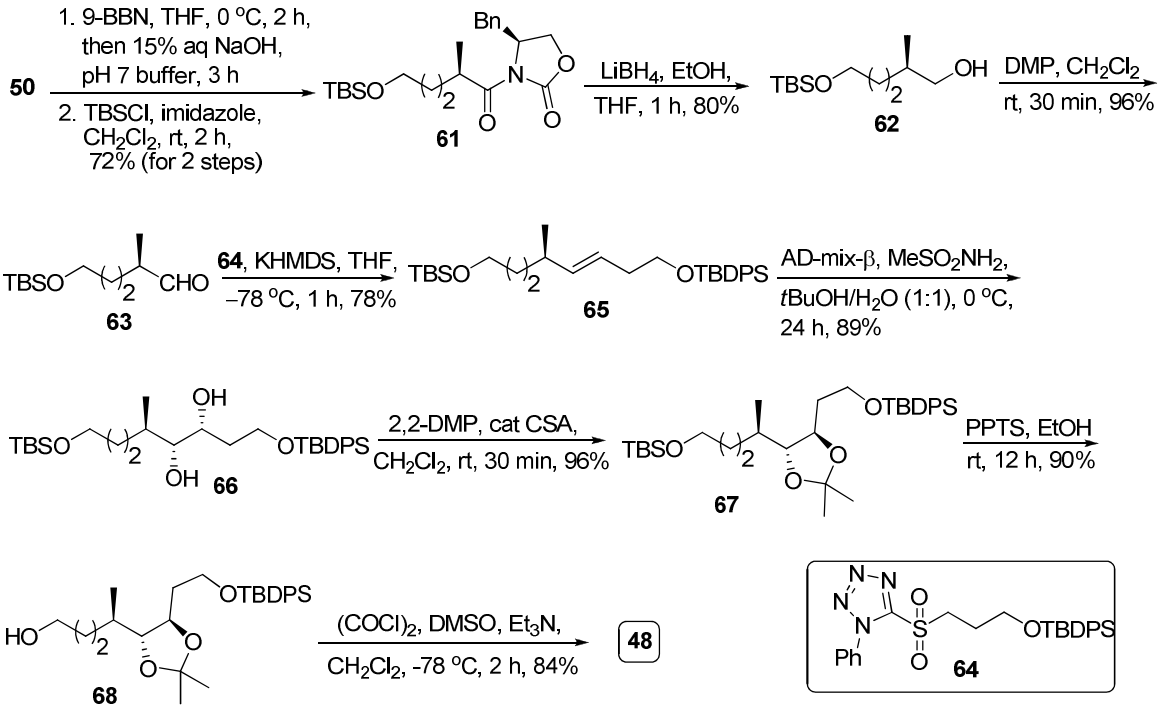


Scheme 1.10

E,E-isomer. Using Sharpless dihydroxylation conditions [$(DHQ)_2PHAL$, $K_3Fe(CN)_6$, K_2CO_3, OsO_4 and $MeSO_2NH_2$], dienoate **52** proceeded to the diol **53** and the resultant diol was treated with triphosgene, Et_3N in CH_2Cl_2 to attain carbonate **54** (94%). $Pd_2(dba)_3{\cdot}CHCl_3/Ph_3P$ catalyzed reduction of **54** at allylic position using mild hydride source (Et_3N/HCOOH) in THF offered *δ*-hydroxy enoate **55** (89%). Silylation of secondary alcohol to **56** followed by ester reduction with DIBAL-H offered allylic alcohol **57** in 84% yield. Stereoselective epoxidation of allylic

alcohol **57** with *m*CPBA afforded a mixture of the *anti*- and *syn*-epoxides in 20:1 ratio, from which *anti*- epoxide **58** could be isolated by column chromatography in 83% yield. Epoxy alcohol **58** was converted to corresponding chloride and treated with lithium diisopropylamide (LDA) in THF provided alkynol **59**. Desilylation of TBS-ether and protection of 1,3-diol as an acetonide **47** using 2,2-DMP and cat. CSA in CH_2Cl_2 in 97% yield.

Synthesis of aldehyde 36:



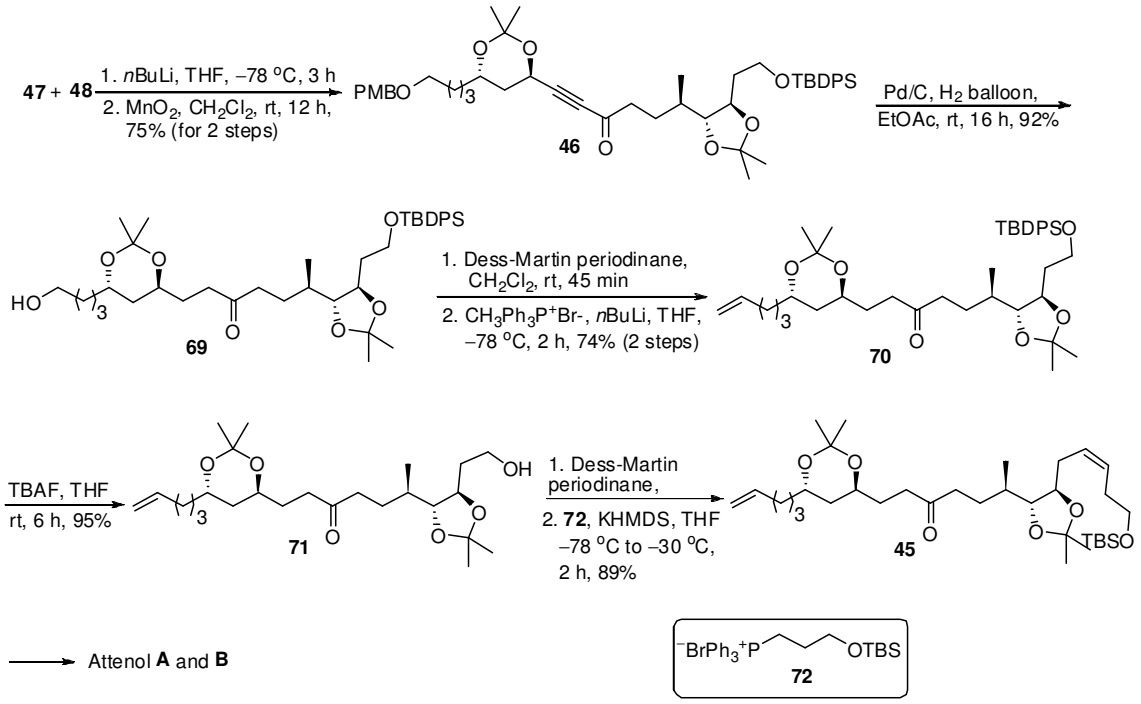
Scheme 1.11:

Hydroboration [9-BBN, pH 7 buffer, 30% H_2O_2] of terminal double bond in **50** offered alcohol, which was immediately silylated as a TBS-ether **61** in 72% yield. Reductive cleavage of chiral auxiliary was achieved with $LiBH_4$ provided alcohol **62.** Oxidation of alcohol to aldehyde **63** and

subsequent Julia–Kocienski olefination with sulfone **64** in presence of KHMDS afforded *E*-olefin **65** as a single isomer (78%). Sharpless conditions (AD-mix-β and $CH_3SO_2NH_2$) employed to **65** to install vicinal diol (**66**) and diol protected as an acetonide (2,2-DMP, cat. CSA in CH_2Cl_2) afforded fully and differentially protected compound **67** (96%). Selective TBS cleavage under mild acidic conditions [PPTS/ethanol] offered the primary alcohol **68** and Swern oxidation of alcohol led to the construction of the aldehyde segment **48** in 84% yield (Scheme 1.11).

Synthesis attenols A and B:

With **47** and **48** in hand, the stage was set to couple these fragments. Coupling of lithiated alkyne (generated from alkyne **47** and *n*BuLi in THF) and aldehyde **48** ended with diastereomeric alkynol, which on MnO_2 oxidation offered alkynone **46** (75% yield). One-pot reduction of alkyne functionality and PMB-ether deprotection using Pd/C under H_2 atmosphere afforded primary alcohol **69** in 92% yield. One carbon homologation of **69** *via* oxidation and Wittig reaction furnished olefin **70** in 74% yield. Desilylation of **70** with TBAF produced primary alcohol **71** in 95% yield. DMP oxidation of alcohol to aldehyde and subsequent *cis*-Wittig reaction with phosphonium salt **72** and KHMDS in THF at −30 ^oC offered acyclic ketone **45** with (*Z*)-olefin in 89% yield. Finally, one-pot global deprotection followed by spiroketalization was achieved with *p*-TSA in methanol to provide the desired spiroketal isomers attenols A and B in 52% and 12% yields, respectively (Scheme 1.12).



Scheme 1.12

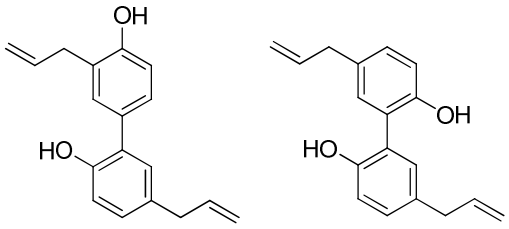
In summary, stereoselective total syntheses of attenols A and B have been achieved using Sharpless asymmetric dihydroxylation, Pd(0)-catalyzed allylic reduction, stereoselective mCPBA epoxidation and Julia-Kocienski olefination reactions as key steps.

CHAPTER V:

This Chapter describes the "Synthesis of neolignan honokiol analogues".

The root and stem bark of the oriental herb *Magnolia officinalis* (also known as *Houpo*) or *Magnoliae obovata* have been used in traditional Chinese and Japanese medicines for treatment of various diseases like flu, anxiety and allergy. Early research on these traditional medicines has identified honokiol **73** and its structural isomer magnolol **74** as

active compounds (Figure 1.4). The structure of **73** consists of a biphenyl skeleton with *ortho, para*-C,C- coupling of *para*-allyl and *ortho*-allyl phenols. Honokiol has demonstrated neurotrophic activity at 0.1 to 1 μM concentrations on the cultures of rat cortical neurons.



Honokiol **73** Magnolol **74**

Figure 1.4:

A SAR study has been already performed on a few compounds where double bonds were reduced and phenols selectively protected. The results revealed that the 4′-phenol and 5-allyl group are essential for neurotrophic activity. As part of our CNS program on the development of novel neurotrophic agents based on natural products new analogues of honokiol as target molecules. We designed honokiol analogues with various substituents at 5′-allyl position. Further, we studied the effect of the substitution of two phenol groups by preparing corresponding methyl ethers (Figure 1.5).

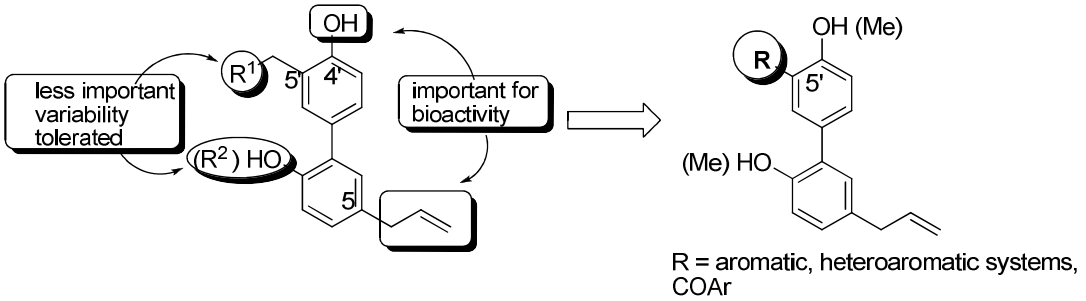
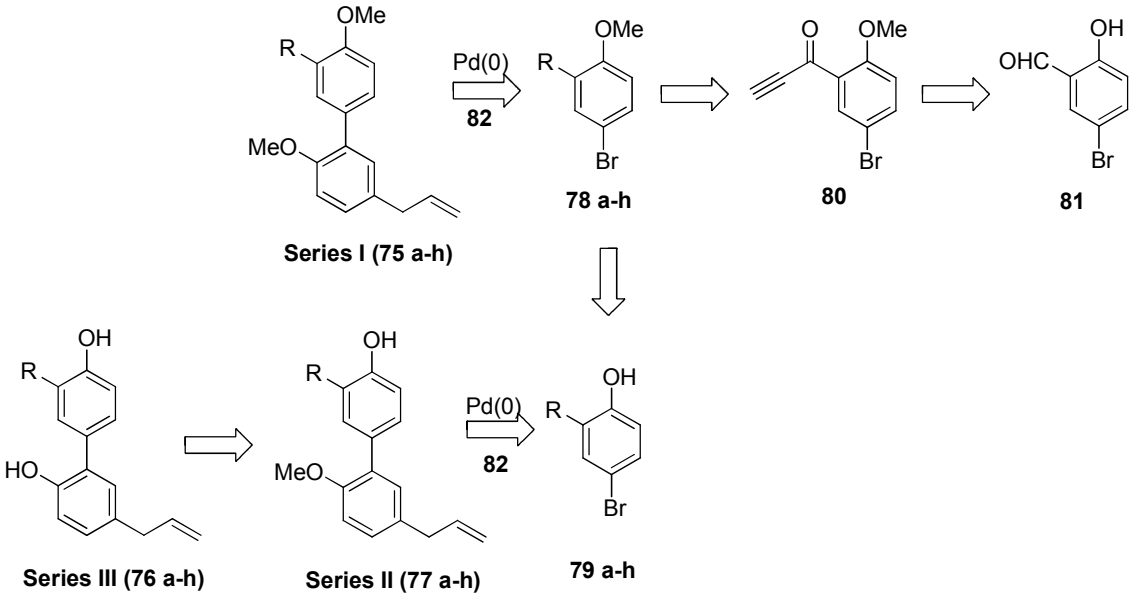


Figure 1.5:

Present Work:

As shown in Scheme 1.13 a convergent and versatile synthetic route to produce designed analogues of **73**, *i.e.,* compounds from Series I, II and III depending upon the number of free phenol groups. Pd-catalyzed Suzuki-Miyaura coupling could be employed to construct biaryls (**75a-h**, **76a-h, 77a-h**) from bromides (**78a-h, 79a-h**) and boronate **82**. The aromatic bromides with various heterocyclic and carbonyl groups at α-position to *O*-methyl/hydroxyl group could be easily obtained from propargyl ketone **80**, which could be synthesized from 5-bromosalicylaldehyde **81**.

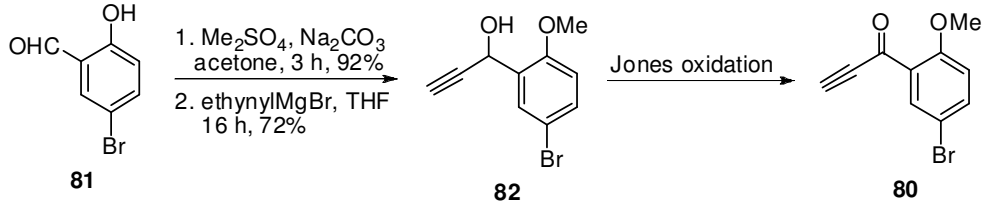


Scheme 1.13: Retrosynthetic analysis

Synthesis of ketone 80:

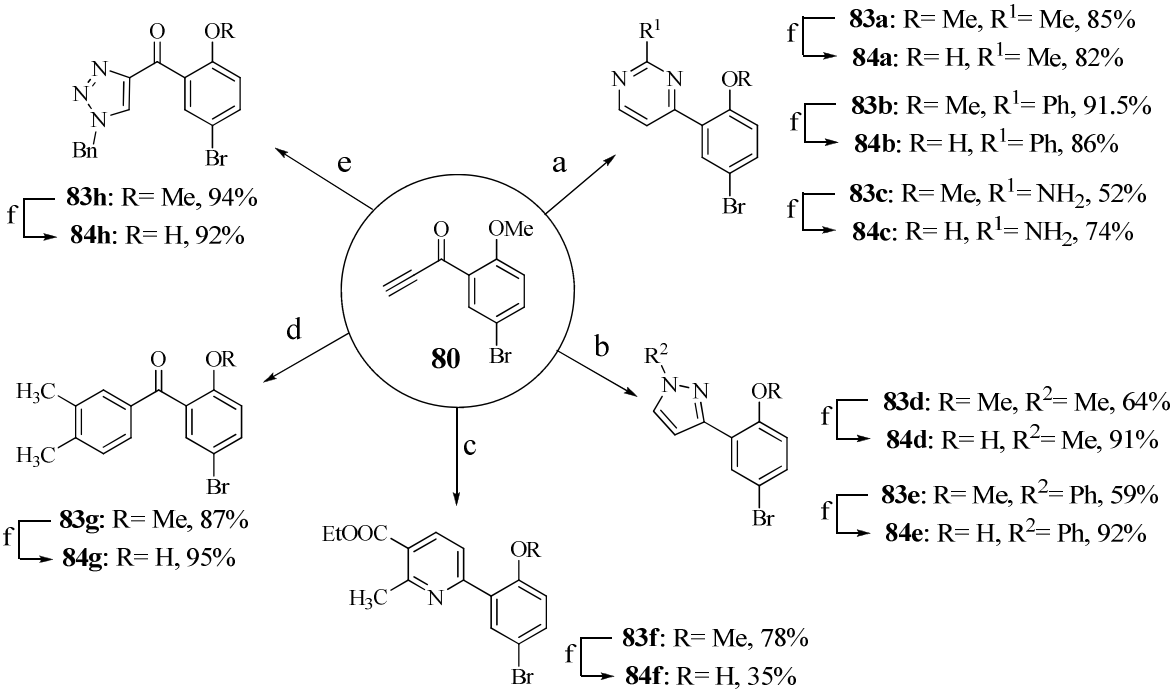
The synthesis of ketone **80** starts with *O*-methylation of 5-bromo salicylaldehyde **81** followed by ethynylmagnesium bromide addition gave

propargylic alcohol **82**. Jones oxidation of alcohol **82** gave the ketone **80** in 78% yield (Scheme 1.14).



Scheme 1.14:

Syntheses of 83a-f and 84a-f:



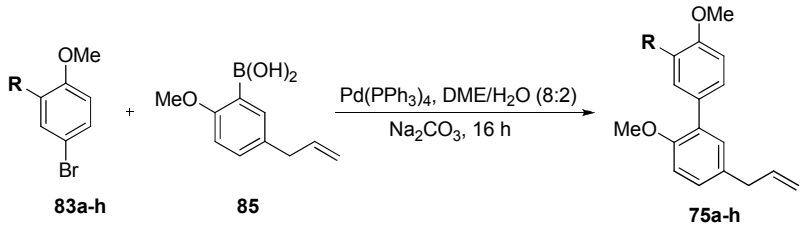
Scheme1.15: *Regents and conditions:* a) $R_1C{=}NH(NH_2).HCl$, Na_2CO_3, CH_3CN, reflux, 8 h; b) $RNHNH_2$, 4 Å MS, EtOH, reflux, 12 h; c) ethylacetoacetate, ammonium acetate, $ZnBr_2$, toluene, reflux, 24 h; d) 2,3-dimethyl-1,3-butadiene, PhMe, 80 ^oC, 24 h; e) benzylazide, EtOH, reflux, 24 h; f) BCl_3.DMS (2M in CH_2Cl_2), dichloroethane, 24 h.

Key intermediate **80** in hand, stage was set for the preparation of the required intermediates. Ketone **80** was refluxed with amidine

hydrochlorides and Na_2CO_3 provided corresponding pyrimidines **83a-c.** Pyrazoles **83d,e** were prepared in excellent yields by refluxing **8** with hydrazines in ethanol for 12 h. The pyridine derivative **83f** was obtained by treating **80** using Bohlmann-Rahtz conditions. Treatment of ketone **80** with 2,3-dimethyl-1,4-butadiene in toluene provided Diels-Alder adduct **83f** in 87% yield. Ketone **80** exposed to benzyl azide in ethanol afforded triazole **83h** (94%). The second series of intermediates **84a-h** were synthesized from demethylation of **83a-h** using $BCl_3.Me_2S$ in CH_2Cl_2 (Scheme 1.15).

Series I analogues:

The series I analogues (**75a-h**) have both phenol functions protected as methyl ethers. The $Pd(PPh_3)_4$ catalyzed Suzuki-Miyaura coupling between aryl bromides **83a-h** and boronic acid **85** and Na_2CO_3 at reflux temperature afforded series I analogues (**75a-h)** in 76-92% yield (Scheme 1.16).



83a-h **85** **75a-h**

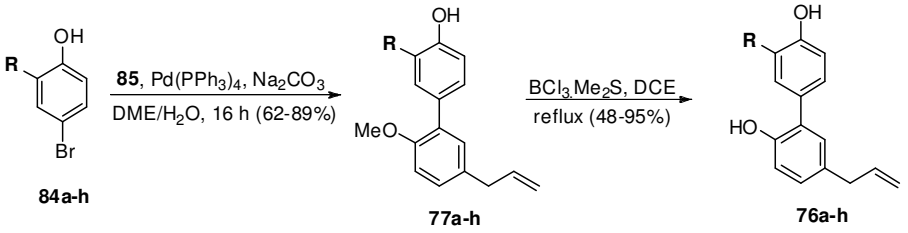
75a (89%)	**75b** (84%)	**75c** (76%)	**75d** (92%)	**75e** (90%)	**75f** (82%)	**75g** (85%)	**75h** (85%)

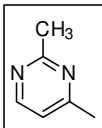
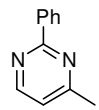
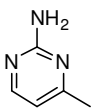
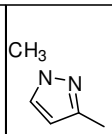
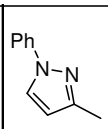
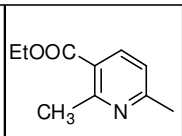
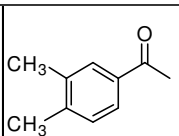
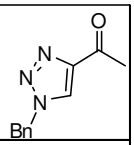
* Numbers mentioned in the brackets are respective isolated yields.

Scheme 1.16:

Series II and III analogues:

The series II derivatives (**77a-h**) were obtained by $Pd(PPh_3)_4$ catalyzed Suzuki-Miyaura cross coupling reaction between aryl bromides **84a-h** and aryl boronic acid **85**. Demethylation of **77a-h** using $BCl_3.Me_2S$ offered series III compounds (**76a-h**) in 48-95% yield (Scheme 1.17 and Table 1.2).



							
77a (82%)	**77b** (79%)	**77c** (72%)	**77d** (89%)	**77e** (85%)	**77f** (62%)	**77g** (86%)	**77h** (81%)
76a (82%)	**76b** (87%)	**76c** (91%)	**76d** (95%)	**76e** (94%)	**76f** (48%)	**76g** (93%)	**76h** (69%)

* Numbers mentioned in the brackets are respective isolated yields.

Scheme 1.17:

In conclusion, an efficient synthetic route affording the analogues of neolignan has been developed. The new analogues were tested against neuro2A cell lines and found that they promote neurite growth moderately.